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Comparison of Effect of 'Valmid'* and Other Hypnotics on Brain Respiration *in vitro*. (22611)

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It has been reported that hypnotics in relatively low concentrations are capable of inhibiting the respiration of cerebral slices during electrical stimulation(1), stimulation due to an excess of potassium or to the addition of 2. 4-dinitrophenol(1,2), and stimulation by the removal of calcium ions(3). Ghosh and Quastel(2) interpreted their data as indicating that the inhibition of brain slice respiration by narcotics is restricted to that aspect of cortical respiration which is stimulated by potassium ions.

This study was undertaken to compare the ability of 'Valmid', a new nonbarbituric hypnotic(4,5,6), to inhibit the potassium-stimulated respiration of slices of brain cortex with that of 'Seconal Sodium'† and that of another nonbarbiturate, urethane. Included in this study was a convulsant barbiturate (sodium ethyl, 1, 3-dimethylbutyl barbiturate), (7) serial No. 09757, which has been shown to be capable of uncoupling oxidation from phosphorylation(9).

Method. All experiments were carried out with the Warburg manometric apparatus. Female albino rats of 150-200 g were sacrificed by decapitation, the brains were quickly removed, and freehand slices were prepared by the method of Deutsch(10). Slices totaling approximately 12 mg dry weight were placed into 2.4 ml Krebs-Ringer phosphate medium containing 0.02 M glucose in the main compartments of the vessel. The hypnotic agent, dissolved in 0.3 ml of 25% polyethylene glycol 200 (PEG 200) made up in Krebs-Ringer phosphate, was added to the vessel contents to give final concentrations equivalent to the anesthetic dose (AD_{50}) or 10 times the AD_{50} of each compound, and a final concentration of 2.5% PEG. The concentration of No. 09757 was based upon its convulsive dose (CD_{50}). A control flask was prepared to contain 2.5% PEG 200. PEG 200 was necessary to keep 'Valmid' in solution throughout the experimental period. The sidearm of each vessel contained 0.3 ml M KCl in Krebs-Ringer phosphate. The center wells contained 0.2 ml 10% KOH. Oxygen was used for the gas phase.

After gassing and thermal equilibration, oxygen uptake was measured for one hour to establish control values. At the end of this period the sidearm contents were tipped into the main compartments and oxygen uptake was measured for another hour. Control experiments showed no difference between first and second hour Qo_2 values. Readings were taken at 15 minute intervals throughout the experiment.

Results. All values in Table I represent the mean values of 8 experiments. Inhibitions were calculated as percentage inhibi-

* 'Valmid' (Ethinamate, Lilly) is ethinylcyclohexyl carbamic acid.

† 'Seconal Sodium' (Secobarbital Sodium, Lilly) is sodium allyl, 1-methylbutyl barbiturate.

TABLE I. Effects of Hypnotic Agents on Normal and KCl-Stimulated Respiration of Rat Brain Cortex. All flasks contained 2.5% PEG 200 unless otherwise noted. The low concentration for each compound is equivalent to anesthetic dose of compound (convulsive dose in case of No. 09757).

Hypnotic	Concentration	Without added KCl		With added KCl (0.1 M)		% stimulation by added KCl (0.1 M)
		Q_{O_2}	% inhibition	Q_{O_2}	% inhibition	
Control	Without 2.5% PEG	12.38	—	21.76	—	76
	With 2.5% PEG	8.75	—	13.19	—	51
'Valmid'	6×10^{-4} M	7.85	10	10.00	24	27
	6×10^{-3} M	.67	92	.93	93	39
'Seconal sodium'	1.5×10^{-4} M	6.28	28	7.15	46	14
	1.5×10^{-3} M	.96	89	1.91	85	99
Urethane	1.4×10^{-2} M	8.94	0	13.58	0	52
	1.4×10^{-1} M	6.67	24	8.77	33	31
No. 09757	5.7×10^{-5} M	8.88	0	12.27	7	38
	5.7×10^{-4} M	1.35	86	1.12	91	0

tions of the control containing 2.5% PEG 200. 'Seconal Sodium', in a concentration equivalent to its AD_{50}, caused a 28% inhibition of the oxygen uptake of "resting" tissue. None of the other compounds appreciably suppressed respiration when employed in this concentration. In the presence of added KCl 'Seconal Sodium' inhibited respiration by 46% and 'Valmid' by 24% when they were employed in concentrations equivalent to their respective AD_{50}'s. Neither urethane at its anesthetic dose nor No. 09757 at its convulsive dose caused a depression of oxygen uptake of either the "resting" tissue or the KCl-stimulated tissue. In concentrations equivalent to 10 times their respective AD_{50}'s (CD_{50} in the case of No. 09757) all compounds suppressed the respiration of both normal and stimulated slices, approaching 100% in the cases of 'Seconal Sodium', 'Valmid', and No. 09757. However there was practically no increase in the amount of inhibition in the potassium-stimulated slices over that in the normal slices by this higher dosage.

The last column of Table I shows the ability of the hypnotic agents to interfere with the ability of the brain slices to respond to stimulation by an excess of potassium ion. In the presence of only PEG 200 there was a 51% increase in oxygen uptake in response to the increase in potassium. When an anesthetic concentration of 'Valmid' or 'Seconal Sodium' or a convulsive concentration of No. 09757 was also added there was a suppression of the response to KCl stimulation. Urethane induced a corresponding inhibition only when present in a concentration equivalent to 10 times its anesthetic dose. At their respective higher concentrations the other agents so completely depressed the normal "resting" respiration that no significance could be attached to the slight changes caused by the potassium addition.

Discussion. The degree of depression of brain slice respiration produced by a drug employed in a concentration equivalent to its anesthetic dose has been proposed as an index of the narcotizing potency of the drug(11). If this concept is applied to the above data, 'Valmid' appears to possess approximately one-half the potency of 'Seconal Sodium', and No. 09757 and urethane exhibit still lesser potencies. The hypnotic-like action of the convulsant barbiturate in this system is not surprising in view of its chemical structure and in view of the findings by Domino *et al.* (8) that it has depressing as well as stimulating actions. Furthermore, Swanson and Chen (7) suggested that the convulsant effect was probably of spinal origin since its occurrence could be prevented by pithing whereas decerebration and medullary destruction were without effect. 'Valmid' at physiological concentrations in this system exhibited an effect which appears characteristic of narcotizing agents, *i.e.*, a slight inhibition of the respira-

tion of normal slices and a significant depression of the oxygen uptake of potassium stimulated slices. This apparent interference of narcotics with respiration is not taken to indicate that the mechanism of their depressant action is an interference with oxidative processes. On the contrary, the decreased oxygen uptake may be a result of an inability of anesthetized brain cortex slices to utilize the products of cellular oxidations, especially in view of the fact that high energy phosphate has been shown to increase during the anesthetic state(12). The depression of the response to potassium stimulation then would be due to a decreased ability of narcotic-depressed tissue to respond to such stimulation rather than to a narcotic suppression of the subsequent increased respiration. In this respect it is noteworthy that Grenell *et al.* (13) were unable to find convincing evidence that the net synthesis of adenosinetriphosphate was a crucial factor in narcosis or that narcotic action was the result of a decreased oxygen uptake.

Summary. 'Valmid', a new nonbarbituric hypnotic, has been compared with 'Seconal Sodium', urethane, and a convulsant barbiturate with respect to its ability to inhibit normal and potassium-stimulated respiration of rat brain cortex slices *in vitro*. In concentrations equivalent to those obtained during anesthesia, only 'Seconal Sodium' depressed the oxygen uptake of normal tissue whereas both 'Seconal Sodium' and 'Valmid' inhibited the respiration of KCl-stimulated tissue.

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A Revised Method for Isolation of *Schistosoma mansoni* Eggs for Biological Experimentation.* (22612)

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Many of the pathologic, immunologic and clinical manifestations of Manson's schistosomiasis appear to be caused by the presence of the egg stage of the parasite in the host tissues(1,2,3,4,5,6,7). Consequently, studies of the egg stage and its pathogens, independently from the cercarial and adult stages, are assuming increasing importance in understanding the disease. We have developed a method of isolating *Schistosoma mansoni* eggs in adequate quantities and in pure enough state to allow for biological assays *in vitro* and *in vivo*. Our method was developed as a modification of comminution and filtration technics(3,8) with the addition of differential sedimentation through media of differing viscosities whereby gross contaminants may be separated and removed.

Materials and methods. Golden hamsters (9) are exposed to approximately 300 cercariae of *Schistosoma mansoni*. Eight weeks after exposure to infection one or 2 hamsters are sacrificed and stored at ordinary refrigerator temperature for 24 or 48 hours. All

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